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MECHANISMS OF HOPPERBURN: An Overview of Insect Taxonomy, Behavior, and Physiology*

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■ **Abstract** Hopperburn is a noncontagious disease of plants caused by the direct feeding damage of certain leafhoppers and planthoppers. Although long studied, especially with *Empoasca* spp. leafhoppers (Cicadellidae: Typhlocybinae), the mechanisms underlying hopperburn have only recently been elucidated. Hopperburn is caused by a dynamic interaction between complex insect feeding stimuli (termed hopperburn initiation) and complex plant responses (termed the hopperburn cascade). Herein we review the nature of the feeding stimuli in hopperburn initiation, especially for *Empoasca* spp., which we also compare with the planthopper *Nilaparvata lugens*. Contrary to previous reports, *Empoasca* hopperburn is not caused solely by toxic saliva. Instead, it is caused by a plant wound response triggered by a unique type of stylet movement, which is then exacerbated by saliva. Electrical penetration graph monitoring has revealed that all *Empoasca* spp. are cell rupture feeders, not sheath feeders, and that certain tactics of that feeding strategy are more damaging than others. Measuring the proportions of the most damaging feeding led to development of a resistance index, the Stylet Penetration Index, which can predict hopperburn severity in different plants or under different environmental conditions and can supplement or replace traditional, field-based resistance indices.

INTRODUCTION

What Is Hopperburn and Why Is It Important?

Hopperburn is a noncontagious disease of plants caused by the direct feeding damage of certain leafhoppers and planthoppers (Hemiptera: Auchenorrhyncha). Insects that induce hopperburn are highly destructive agricultural pests worldwide, causing millions of dollars' worth of yield loss and control costs. Hopperburn from the feeding of the brown rice planthopper, *Nilaparvata lugens* (Stål), is the primary cause of in-field yield loss to rice throughout Asia (81). The white-backed planthopper, *Sogatella furcifera* (Horvath), also causes direct feeding damage to rice and is especially important in Japan (11, 81). *Empoasca kraemeri* Ross and Moore is the most important pest on common bean in Latin America, causing up to 95% loss in yield in unmanaged plantings during the dry season (26, 74) and inestimable economic privation to small-scale farms. Its North American congener, the potato leafhopper, *Empoasca fabae* (Harris), is the most severe pest on alfalfa in the midwestern and eastern United States (52). The damage caused by *E. fabae* to northeastern alfalfa fields cost $53 million in 1987 alone (44). *Amrasca devastans* (Distant) is the most economically important pest on cotton in India (2). Several other hopperburning auchenorrhynchans are major pests on crops worldwide.

The insect- and plant-related mechanisms of hopperburn have been studied for nearly 100 years, yet very little is known outside of the *Empoasca*-legume systems. The feeding of *E. fabae* on (primarily) alfalfa has been the most-studied model system worldwide. A 1978 bibliography of *E. fabae* (27) lists 1676 papers, many of which involved hopperburn causation. Yet, until recently, a holistic understanding of hopperburn mechanisms has eluded scientists. Research performed in the 1920s through the 1940s to define the disease and its causes became mired in controversy. By the 1950s, significant progress had ceased and the cause of hopperburn became known as an intractable problem.

Many conclusions from this historical hopperburn research are either incomplete or incorrect. Therefore, except in the important case of Smith & Poos (69), this paper does not review the historical research. *E. fabae* research up to 1989 was reviewed in Reference 35. However, much research has been undertaken in the 15 years since that review, work that finally has unraveled the riddle of hopperburn and its cause.

Objective of This Review

Hopperburn is caused by a highly dynamic interaction between complex insect stimuli and complex plant responses. We term all the insect feeding stimuli to the plant *hopperburn initiation*. These stimuli involve not only the toxicity of insect saliva, as has often been stated in the literature, but also unique stylet activities that cause plant cell wounding. We term all the dynamic, sequential, and cumulative responses of the plant to these stimuli *the hopperburn cascade*.

The objective of this paper is to review the past 20 years of research on hopperburn initiation. Unless otherwise stated, discussion centers on the best-studied *Empoasca* model systems: *E. fabae* (Harris) on alfalfa, and *E. kraemeri* Ross and Moore and *E. abrupta* DeLong on common bean. When appropriate, inferential comparisons also are made for *N. lugens* and other hopperburning species.

A companion paper in preparation will provide, for the first time, a similarly comprehensive review of research on the plant responses comprising the hopperburn cascade. It will review research on the anatomical and systemic, whole-plant physiological responses to *Empoasca* feeding. It also will include logical inferences of plant biochemical and molecular mechanisms that may be involved in the cascade, drawn from the literature on other study systems. It is our goal that these two companion papers form the most in-depth and integrated review available on hopperburn, the most important model system for the cause of direct feeding damage by phytophagous hemipterans.

SYMPTOMS AND TERMINOLOGY OF HOPPERBURN

General symptoms of hopperburn, which often resemble senescence, occur in all accounts of hopperburn no matter which species is affected. These include (*a*) tip-wilting in very young plants, (*b*) leaf chlorosis (yellowing or other leaf colors) expanding to engulf the leaf, followed by premature leaf drop (in older plants), and (*c*) plant stunting, usually caused by reduced stem elongation.

Many plants additionally display more specific symptoms. For example, on alfalfa and certain clovers *Empoasca* spp. cause a unique triangular yellowing at the tips of leaves (1). The yellow coloration eventually engulfs the entire leaf and sometimes deepens to a red or purple, but necrosis of leaves seldom occurs. Sudden leaf drop is usually not preceded by loss of turgidity. In contrast, many other plants show distortions of leaf shape and leaf necrosis. For example, *E. fabae* feeding on potato (24), soybean, and common bean causes early marginal leaf chlorosis followed by necrosis that forms an expanding front, engulfing the leaf from outer edge to inside. Chlorosis is accompanied by strong, downward leaf curling and, just before leaf drop, loss of turgidity (75). These species-specific symptoms often have led to confusion in understanding their cause.

Although chlorosis is more visible, the most important symptom for yield reduction is reduced growth (termed stunting) and reproduction. In forage crops such as alfalfa, for which yield is vegetative matter, stunting directly and proportionally leads to reduced yield. In crops such as common bean or rice, for which reproductive output is the yield, stunting disproportionately leads to reduction in yield.

Until recently, there was confusion in the literature regarding the definition of hopperburn, particularly that caused by *Empoasca* spp. Originally, hopperburn referred only to chlorosis symptoms, also called potato leafhopper yellows

(35), while reduced growth was termed potato leafhopper stunting. Then, alfalfa breeders developed genotypes that exhibited reduced or no chlorosis but still became stunted. This was one of the first clues that chlorosis was an indirect, secondary response to *E. fabae* feeding. We advocate that all systems use the broader, modern definition of hopperburn stated above because, even if not all possible symptoms are displayed, the disease is caused by the same underlying plant mechanisms in response to the same initiating insect stimuli.

INTRODUCTION TO ELECTRICAL PENETRATION GRAPH MONITORING

The behavioral mechanism of hopperburn was controversial for nearly 50 years in large part due to technological limitations. The clear solution came with the advent of modern means of studying hemipteran feeding, especially electrical penetration graph (EPG) (i.e., electronic) monitoring of insect feeding. EPG was originally developed by McLean and Kinsey (54–56), with significant enhancement by Tjallingii (72), to detect when, where, and how a hemipteran inserts its stylets into plant tissues and performs behaviors within them. This process is termed probing or stylet penetration (77). Thus, for the first time scientists could objectively observe the location and behaviors of identified probes, independent of salivary sheaths or plant damage. The principle and method are simple and have been extensively reviewed recently (22, 77). Two major systems (AC and DC) of EPG monitoring, with many different designs, have been used over the years (77). But all are the same in broad principle. Briefly, the test insect is attached to the monitor through a gold wire glued to its dorsum with conductive paint. A low-voltage current is introduced into the test plant via an electrode to the soil or plant's tissues. When the insect's stylets penetrate the electrified plant, the circuit is closed. Changes in voltage across the stylets (due primarily to the electrical resistance of fluids flowing through them in the AC system, but also to plant or fluid-flow biopotentials in the DC system) (77) are amplified and recorded as a time-varying waveform for later measurement and analysis.

AC and DC monitors allow quantification of the duration and number of probes (the periods of time when the stylets are inserted into the plant). More importantly, however, the appearance of waveforms within a probe can be correlated with many types of probing behaviors, including various types of stylet movements, salivation, ingestion, manner of stylet penetration (i.e., inter- versus intracellular), and cell types punctured. Generally, the DC system provides more waveform detail than does the AC, owing to its detection of biopotenial as well as resistance components (77).

Most studies of auchenorrhynchans to date have been performed with the AC system (see Reference 5 for all works to 1990). The DC exceptions are an initial waveform characterization study of *N. lugens* (41) and an elegant waveform

correlation study of *Cicadulina mbila* Naude (45). All studies of *Empoasca* spp. to date have used the Missouri-type AC monitor (6).

N. lugens is the only auchenorrhynchan species that has been recorded with DC systems as well as AC (40, 41, 47, 76). Interestingly, many of its waveforms are remarkably similar in appearance at the medium- to coarse-structure level, regardless if the AC or DC system was used (5, 30). This is in striking contrast to AC versus DC waveforms from sternorrhynchan species such as aphids, for which the two types are drastically different and difficult to correlate without elaborate experiments (77). This similarity among auchenorrhynchan waveforms is probably due to the intracellular penetration style of auchenorrhynchan stylets (which presumably destroys plant membrane biopotentials) as opposed to the intercellular style of sternorrhynchans (which preserves biopotentials). The major difference between *N. lugens* AC and DC signals is that DC waveform fine-structure can distinguish phloem from xylem ingestion (41), whereas the AC system has not been demonstrated to do so (76).

TAXONOMY OF HOPPERBURNING SPECIES

Table 1 (follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org) lists the most important hopperburning pest species worldwide. All 23 belong to the hemipteran suborder Auchenorrhyncha, and 19 of the 23 species are leafhoppers (Cicadellidae) in the subfamily Typhlocybinae. Within Typhlocybinae, 16 of 19 documented hopperburning species belong to the tribe Empoascini, especially the genus *Empoasca* and related genera. These *Empoasca*-like species (Table 1) are taxonomically challenging to study; their systematics is currently undergoing change, and their phylogeny is poorly known. Only three known hopperburning species are from the typhlocybine tribe Erythroneurini (Table 1). Interestingly, one species from the subfamily Nirvaninae, *Sophonia rufofascia* (Kuoh & Kuoh), causes hopperburn (37). Although nothing is known of this species' feeding behavior, Nirvaninae is in the same phylogenetic lineage as Typhlocybinae (16). Given the behaviors discussed below, and the large number of nonburning typhlocybine species, it seems likely that the unique behaviors triggering hopperburn are highly derived traits. Future study of typhlocybine and nirvanine feeding might contribute to improved taxonomy and phylogeny.

Only four species of planthoppers (Delphacidae) are known to cause hopperburn (Table 1). These include *N. lugens* and *S. furcifera*. This paucity is probably an artifact of little study. In fact, there are hints that other planthopper species cause hopperburn but nothing is known of their feeding biology, e.g., *Unkanodes sapporonus* (Matsuda) (Table 1).

Finally, knowledge of host plant range also may have phylogenetic value. All typhlocybine-empoascines and nirvanines that cause hopperburn feed on dicotyledonous plants; all typhlocybine-erythroneurines and planthoppers that do so feed on monocotyledonous plants (Table 1).

FEEDING BEHAVIOR ASSOCIATED WITH HOPPERBURN

Hemipteran Feeding Strategies

In 1972, Miles (57) organized many observations in the literature by proposing that all hemipterans use one of two different feeding strategies. These were (*a*) sheath feeding, in which insects seal their stylet tips into (typically) a vascular cell via a sheath made of solidifying saliva, and (*b*) lacerate-and-flush feeding, in which a sheath is not made and stylets move continuously or intermittently, lacerating cells, secreting watery saliva, and ingesting the resulting slurry of cell contents. As explained further below, we propose herein to rename the latter strategy cell rupture feeding and refer to it hereafter as such. Understanding which of these strategies is used by typhlocybines has been a crucial part of discerning the mechanism of hopperburn initiation.

Typhlocybine Feeding Strategy: Stipplers Versus Burners

By the early twentieth century, it was recognized that most auchenorrhynchan species employ the sheath feeding strategy. However, it was also recognized that most typhlocybine leafhoppers feed differently because they cause an unusual and characteristic symptom on leaves: round, silvery-white marks called stipples (69, 80). This group of typhlocybines hereafter is called the stipplers, with *E. abrupta* and *Zyginidia scutellaris* Herrich-Schaeffer as examples. At the same time, several other species (Table 1) were recognized as not causing stipples and to be associated with hopperburn symptoms. This group hereafter is called the burners, with *E. fabae* and *E. kraemeri* as the model species.

In their landmark historical paper, Smith & Poos (69) histologically compared cellular plant feeding damage from six *Empoasca* species fed alfalfa or clover. They found that all five stippler species, including *E. abrupta*, caused removal of cell contents in leaf interveinal and parenchyma/mesophyll tissues (but no effects to vascular tissue) and left no salivary sheaths. The authors (69) concluded that these species ingested the contents of mesophyll cells without making salivary sheaths, i.e., they were cell rupture feeders.

Smith & Poos (69) also found that *E. fabae*, the only burner species they studied, did not cause significant removal of cell contents in alfalfa. Although mesophyll cells near the vascular tissue were "emptied of their contents," their destruction was not as severe as that from the other species' feeding. On the basis of finding a few, tenuous salivary sheaths, Smith & Poos (69) concluded that *E. fabae* was a sheath feeder that ingested from phloem because the sheaths terminated in phloem tissue. For the next 55 years, it was thought that typhlocybine leafhoppers use one or the other feeding strategy, depending on the species, and that hopperburn was caused by sheath feeding and not cell rupture feeding.

Modern research now strongly supports the view that all *Empoasca* spp. (both stipplers and burners) use primarily the cell rupture feeding strategy and do not make true salivary sheaths (38, 62). This conclusion was first suggested by early

EPG monitoring of *E. fabae* by Hunter, Wayadande, and Backus (4, 36, 79) that revealed waveforms different from those seen before with any previously studied, sheath-feeding leafhopper. Backus (5) also showed that *E. fabae* nymphs consume chlorophyll, indicating ingestion of whole mesophyll cell contents. The amount consumed varied among host plants and was especially high on broad bean but low on alfalfa. This was the first indication that *E. fabae* sites of ingestion vary from plant to plant, as do settling sites (7).

Kabrick & Backus (38) then tested the fundamental premise of Smith & Poos (69), i.e., whether *E. fabae* leaves a salivary sheath with every probe, as do all true sheath feeders. Using EPG to identify probes and correlate them with histology of the probed plant tissue, they found that only 87 (24%) of 368 identified probes left any kind of sheath saliva deposit, which were either tenuous sheaths or amorphous, disconnected globular deposits. This is similar to the absence of full salivary sheaths in probes by *Eupteryx melissae* Curtis, a typhlocybine stippler (tribe Erythroneurini), whose probing on sage (*Salvia officinallis*) was studied without EPG by Pollard (63). Thus, without the benefit of EPG, Smith & Poos (69) had been unable to detect three quarters of the feeding their test *E. fabae* had performed. The loss of that critical information meant that they could not identify the major stimuli initiating hopperburn. Only three probes (0.8%) of Kabrick & Backus (38) were correlated with something resembling a true salivary sheath (although they are now termed pseudosheaths). Interestingly similar to the findings of Smith & Poos (69) and other early works, all three sheaths terminated in the phloem. These findings led Kabrick & Backus (38) to reject the conclusion of Smith & Poos (69) that *E. fabae* is a sheath-feeder, although they agreed that *E. fabae* ingests from phloem. However, it does not do so from a single sieve element cell using the sheath strategy. Instead, *E. fabae* ingests from multiple, general phloem cells in a vascular bundle via the cell rupture feeding strategy. This was the first time that performance of this strategy was confirmed in vascular tissues, and by a burner typhlocybine. Therefore, the difference in plant symptoms triggered by these species (i.e., stippling versus burning) was not caused by a difference in feeding strategy.

Stylet Penetration Tactics of Stipplers and Burners

It is known now that *Empoasca* spp. are plastic in their feeding behavior and perform a repertoire of three different feeding substrategies, or stylet penetration tactics (67) (Figure 1). Tactics are defined as stereotypical sequences of probing behaviors (best represented by EPG waveforms) within a feeding strategy. This ability has not been demonstrated for any other hemipteran studied to date. Short-duration probes (under 4 to 10 min) by *Empoasca* spp. can usually be unambiguously assigned to a single tactic (67; A.S. Al-Dawood & E.A. Backus, unpublished data). However, long-duration probes (>10 min) often start in one tactic, and then switch to a second or sometimes even a third in mid-probe (62, 67). Multiple lines of evidence obtained by videotaping and EPG monitoring of feeding behavior, and histological and statistical analysis (18, 21, 38, 62, 65–67), support the following conclusions about these tactics.

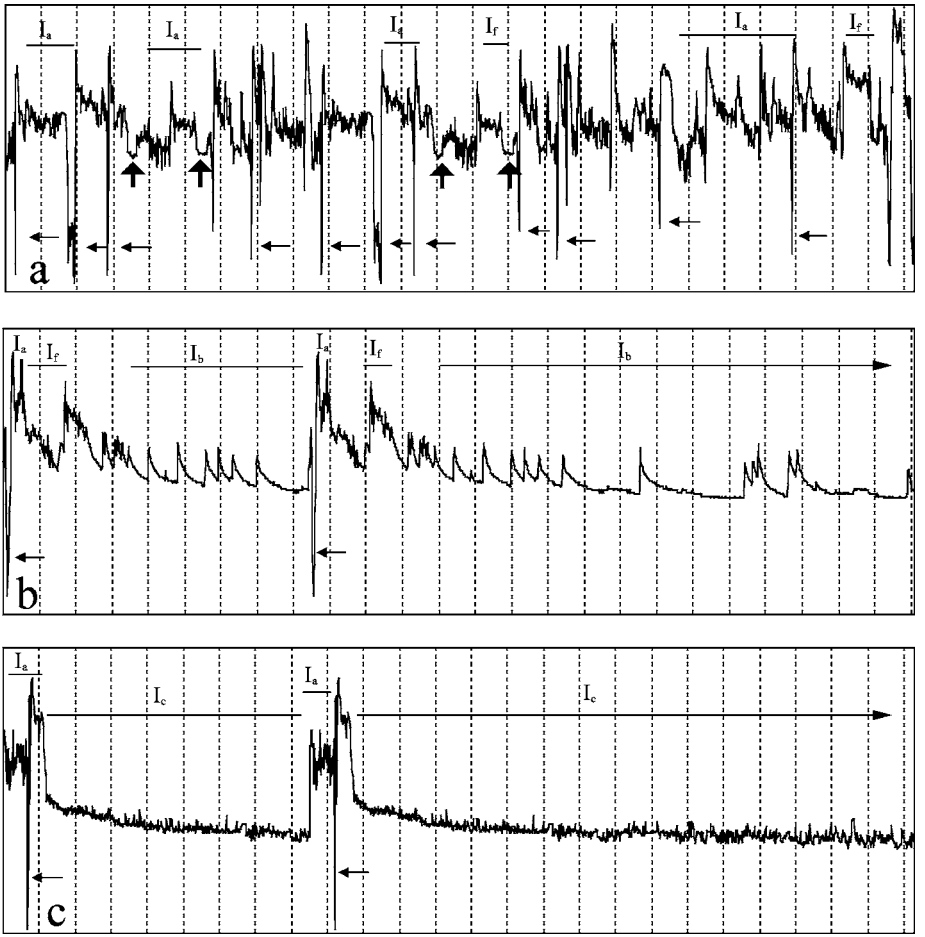

Figure 1 Waveforms from stylet penetration tactics performed by *E. kraemeri* while electronically monitored on *Phaseolus vulgaris*. Small arrows point to the start of probes. Vertical arrows show short durations of I_c (ingestion with stylets motionless). Vertical divisions are 1 min each. (*a*) Pulsing laceration. (*b*) Lacerate-and-flush. (*c*) Lance-and-ingest. Figure from Reference 65.

First, both burner and stippler *Empoasca* spp. possess almost the same behavioral repertoire of three tactics. Each tactic has a dominant EPG waveform. These hallmark waveforms can be performed in any other tactic for short durations, but each one is performed for much longer durations in its own tactic. Second, the major behavioral difference between stipplers and burners is which variant of one of the tactics (lacerate-and-sip) is performed, the length for which it is performed, and in which tissue(s). Third, burner species have the ability to vary the degree of performance of all three tactics on different host plants; it is not known whether

stipplers have this capability. Fourth, each tactic causes different types and severity of hopperburn symptoms. Descriptions of the tactics and the evidence for these conclusions are as follows.

LACERATE-AND-SIP This tactic's hallmark EPG waveform is I_a (for ingestion type a) (65, 67) (Figure 1*a*). In retrospect, the I_a waveform was poorly named, because it represents primarily secretion of watery saliva, during continuous steady in-and-out movements of the stylets (i.e., laceration). A small amount of ingestion probably occurs simultaneously. The stylets intracellularly slice through and ingest the contents of multiple columns of cells (8, 36), a behavior also termed multiple cell laceration (8) or channel cutting (3). This tactic has been best studied in *E. fabae* and *E. kraemeri*.

Evidence supports that only disconnected deposits of sheath saliva are secreted during I_a (38). No true salivary sheaths are made. More than 20 stylectomies were performed during I_a by *E. fabae* on alfalfa (X. Ni & E.A. Backus, unpublished data). In each case, the wired insect was allowed to probe until it performed I_a, and then it was rapidly CO_2-anesthetized and stylectomized via electrocauterization. Only three stylet bundles were intact following histological preparations (performed as in Reference 20). Figure 2 shows that no sheath saliva (which would have stained bright red) surrounds the bare stylets, which explains why stylets were usually dislodged during tissue preparation. Figure 2*b* also confirms that *E. fabae*, like all other leafhoppers, uses the mandibular stylets ahead approach during intracellular stylet penetration (3a). However, unlike sheath feeding leafhoppers, *E. fabae*'s maxillary stylet tips are splayed far apart during probing, similar to those of the cell rupturing stippler *E. melissae* (63). This implies very deep protraction and retraction of the stylets during laceration, and also a trough-like aperture to the food canal, which may aid ingestion and/or watery salivation. The stylets shown are at the beginning of the planar arc (described below) that cuts through the vascular bundle. Cell emptying from active laceration and ingestion occurs in a fan-like area interior to the maxillary stylets (Figure 2*b*).

There are two variants of lacerate-and-sip. The first, pulsing laceration, is composed of bouts (lasting 20 min to several hours) of many repeated, short-duration probes. Each probe lasts about 1 to 2 min for *E. kraemeri* (67) (Figure 1*a*) or 2 to 6 min for *E. fabae* (A.S. Al-Dawood & E.A. Backus, unpublished data). Pulsing laceration is performed primarily in or near vascular tissues in stems and veins (12, 79). Many of its probes can be composed entirely of I_a. However, frequently the stylets cease movement, and a second waveform, I_c (for ingestion type c), occurs for 10–90 sec at a time (4, 12, 67) (Figure 1*a*). This waveform represents continuous ingestion with stylets motionless, probably of lacerated cell contents when so brief (38). After this brief interlude of I_c ingestion, the stylets again move vigorously in I_a.

During pulsing laceration, the stylets move in an unusual manner, starting straight outward from the insect's body, perpendicular to the plant surface. The burner species usually start a probe by arcing the stylets strongly to one side (as in Figure 2), and then moving in and out steadily center-ward, eventually arcing

strongly to the other side. In this manner, the stylets move in a planar arc, cross-cutting a vascular bundle in stem, petiole, or leaf vein. Once an arc is complete, the stylets are withdrawn and the insect takes one or two steps forward along the vascular bundle, immediately reinserts its stylets, and begins to slice another arc of channels (36). This style of repeated short, lacerating probes greatly wounds vascular cells, lacing them with saliva that is not reingested during the brief ingestion events. Pulsing laceration into vascular tissue is performed exclusively by the burner species. Evidence supports that pulsing laceration is the main behavior causing burning by *E. fabae* and *E. kraemeri*.

A second variant of lacerate-and-sip is described herein for the first time. Sawing laceration is not pictured but closely resembles pulsing laceration (Figure 1*a*). I_a and I_c are again alternately performed, with the same stylet activities correlated. But in this case, the insect's body remains motionless in one location for long-duration probes (15 to 60 min); each waveform event can be 3 to 10 min long. Virtually identical waveforms with long durations by *Z. scutellaris* were AC EPG-monitored by Marion-Poll et al. (53). I_a was termed A (or A′) and I_c was termed R (53). Sawing laceration is performed by *E. abrupta* (E.A. Backus, unpublished data) and *Z. scutellaris* (53) primarily interveinally on leaves, avoiding vascular tissues. Stylets of both *E. melissae* and *E. abrupta* penetrate parallel to the leaf surface (63; E.A. Backus, unpublished data) so that they saw a series of channels that are arrayed radially (via labial rotations) all around the stylet entry point. The rosette of channels formed becomes the basic structure of a stipple. In some plant species, the green leaf tissues between the branches of the rosette bleach within days after feeding, leaving a white circle as the stipple (E.A. Backus, unpublished data).

Interestingly, sawing laceration also is performed by both burner species when they are artificially confined to a small space on the plant (62). Under such circumstances, *E. kraemeri* and *E. fabae* probe both vascular and nonvascular tissues (62; E.A. Backus, unpublished data). However, they do not leave a stipple mark as does *E. abrupta*, probably in part because the duration of their probes is not as long (62). In contrast, *E. abrupta* has not, to date, been observed to perform pulsing laceration (E.A. Backus, unpublished data). Stippling behavior is probably not due solely to sawing laceration. *E. abrupta* and *Z. scutellaris* (53) perform at least one of the other tactics, usually lacerate-and-flush, during most long stippling probes.

N. lugens does not appear to perform any waveform or probing behavior that is analogous to I_a laceration. Like most delphacids, *N. lugens* is a sheath-feeder (40) that makes moderately long (minutes to hours in duration) probes. Highly compressed views of *N. lugens* S waveform appear similar to I_a (30). However, correlations in artificial diet, which allows a clear view of stylet movements, have not been performed. Given that true sheath secretion seldom allows active stylet laceration, it seems unlikely that *N. lugens* performs this behavior.

LACERATE-AND-FLUSH The lacerate-and-flush tactic was previously called cell rupturing (67) (Figure 1*b*). This tactic closely resembles Miles' (57) classical description of the lacerate-and-flush feeding strategy. It is composed of

medium-to-long duration events that vary from about 5 to 30 min, depending on the species (36). Lacerate-and-flush also has a hallmark waveform, I_b (ingestion type b), that always follows a few seconds to minutes of I_a laceration. I_b has been correlated with stylets that are motionless or making only slow, steady movements intracellularly (but seemingly in random directions and angles in the plant), plus secretion of spurts of (mostly) watery saliva, alternating with ingestion of both cell contents and previously secreted, stainable saliva (12, 36, 62). Because excretory droplets rarely occur during I_b, it had not been demonstrated to be a type of ingestion, until Njihia (62) showed that duration of I_b was directly correlated with the degree of cytoplasmic removal in mesophyll/parenchyma cells, and indirectly correlated with the number of stainable salivary deposits left behind. These combined actions resemble Miles' (57) description of flushing. Therefore, we are herein redescribing the I_b waveform as flushing to replace its previous description as single-cell puncturing (36).

Comparison of EPG recordings and histology from *E. abrupta* and *E. kraemeri* reveals that durations of the laceration and flushing phases of this tactic are strikingly different between stipplers and burners (E.A. Backus, unpublished data). *E. abrupta* performs much longer durations of both phases during long probes than *E. kraemeri* does, and in primarily mesophyll nonvascular leaf tissues. *E. kraemeri* performs shorter durations in short-to-medium probes that can occur in either vascular or nonvascular tissue.

A waveform almost identical to I_b (often termed R) has been electronically monitored from many other nontyphlocybine auchenorrhynchans (both sheath-making and non-sheath-making) by AC systems. However, stylet activity correlations could not be determined in most studies, although stylet locations (when studied) usually were in the mesophyll (45, 78; and references and discussions therein). Authors have defined this waveform as stylets at rest (78) or stylets at work (45). We hypothesize that these I_b-like waveforms represent flushing behavior (i.e., alternating watery salivation and ingestion) in all species from which they are recorded. If flushing is performed by sheath-feeders, it may occur through a salivary sheath and without preceding laceration. In contrast to nontyphlocybine leafhoppers, *N. lugens* and *S. furcifera* do not appear to exhibit this flushing behavior, unless the enigmatic DC Pattern 6 of Kimmins (41) might prove in future studies to be an I_b-like behavior.

LANCE-AND-INGEST This tactic was previously termed lancing sap ingestion (67, 68). It is composed of long probes (by *Empoasca* standards), 10 to 40 min, performed in vascular tissues on stems and veins (12, 38). The hallmark waveform of this tactic is long (>2 min) events of "spikey" I_c (38, 67) (Figure 1*c*).

This type of I_c is correlated with continuous production of clear excretory droplets (62), an indication of vascular ingestion in all hemipterans, as well as salivary pseudosheaths, i.e., continuous, linear accumulations of semigelling saliva that resemble true sheaths (38, 62) but are not persistent in the plant (19; see further discussion below). Because they invariably terminated in the phloem (62), these

pseudosheaths are probably what both Smith & Poos (69) and Kabrick & Backus (38) observed.

Waveforms for this tactic somewhat resemble the waveforms of typical sheath-feeding auchenorrhynchans. For example, the AC I waveform of *N. lugens*, like most auchenorrhynchan ingestion waveforms, superficially resembles "spiky" I_c in coarse structure. It has been correlated with both excretory droplets (30) and salivary sheath termini in vascular tissues (40).

Lance-and-ingest probably represents direct ingestion of phloem sap from "leaking" sieve elements (65). We suspect that, during the more frequent process of laceration, the leafhopper's stylets occasionally lance a phloem sieve element, which then leaks phloem sap for a certain amount of time. This may be possible because putative anti-phloem-blockage factors, such as carbohydrases and proteases in the watery saliva, might temporarily prevent callose or P-protein buildup. The insect then, opportunistically, ingests the sap with stylets motionless. Eventually, the sieve element plugs up and the flow of fluids ceases. The insect then secretes gelatinous sheath saliva as it withdraws the stylets, similar to the way a sheath-feeding leafhopper secretes true sheath saliva to fill the sheath upon stylet withdrawal (45, 55). This is the most logical explanation for the unusually narrow, seemingly intercellular appearance of these pseudosheaths and their invariant location in the phloem (62). Table 2 summarizes the biological meanings of these stylet penetration tactics and their variants.

Revisiting Feeding Strategies and Their Evolution in Light of Stylet Penetration Tactics

A specific sequence of EPG waveforms (i.e., a stylet penetration tactic) is similar to Miles' (57) description of lacerate-and-flush feeding. Yet, if this style of feeding were the true strategy of *Empoasca* leafhoppers, it would be the exclusive probing behavior performed. Instead, lacerate-and-sip (either pulsing or sawing laceration) represents the bulk of the probing durations for most species, and it clearly does not belong to the sheath feeding strategy. We therefore propose that the term lacerate-and-flush be demoted from a strategy to a tactic, and that the term cell rupture be promoted from a tactic to a strategy. This is the terminology used herein. Thus, the cell rupture feeding strategy is composed of at least two tactics and their variants: lacerate-and-sip (either pulsing or sawing laceration) and lacerate-and-flush.

Lance-and-ingest appears to be a transitional behavior between the ancestral sheath feeding strategy of most auchenorrhynchans and the cell rupture feeding strategy of the highly derived Typhlocybinae (60). It is not composed primarily of either laceration or flushing and causes only minimal cellular disruption. Nonetheless, this tactic fits slightly better in the cell rupture feeding strategy than in the sheath feeding strategy because insects that perform this behavior probably neither secrete a solid salivary sheath along the stylet pathway, nor use it to seal the

TABLE 2 Summary of the stylet penetration tactics of *Empoasca* spp. leafhoppers, their EPG waveforms, and characterization

Stylet penetration tactic or variant of tactic	Hallmark waveform	Biological meaning of waveform	Behavior during tactic
Lacerate-and-sip	I_a	Rapid stylet protraction/ retraction with simultaneous watery salivation or brief ingestion, occasional salivation of disconnected sheath deposits.	Active laceration/wounding of plant tissues, no complete salivary sheaths.
Pulsing laceration			Bouts of contiguous, short probes closely spaced on plant. Stylets cut planar channels in each probe that cross-section the area of a vascular bundle, perpendicular to plant surface. Performed only by burners.
Sawing laceration			Long-duration probes on leaves. Radial rosette of stylet channels cut parallel to plant surface, in mesophyll-parenchyma cells. Performed mostly by stipplers, or burners under stress.
Lacerate-and-flush	I_b	Flushing; stylets motionless or advancing slowly, alternating watery salivation and ingestion.	Medium-to-long-duration probes beginning with brief laceration followed by flushing. Usually in mesophyll-parenchyma cells, sometimes in vascular cells. On some plants, can puncture and drain individual mesophyll cells. Performed by stipplers and burners.
Lance-and-ingest	Spiky I_c	Ingestion with stylets motionless; clear excretory droplets.	Medium-to-long-duration probes beginning with brief laceration followed by ingestion. In vascular tissues, often leaving a pseudosheath behind upon withdrawal of stylets. Performed by stipplers and burners.

stylets into an ingestion cell. Also, although both stipplers and burners perform lance-and-ingest, it is not the primary tactic of either group; it is employed much less often. Thus, we hypothesize that lance-and-ingest is an evolutionary remnant, with the sheath filling that occurs at the end being a vestigial behavior occasionally triggered by the direct ingestion of phloem sap.

Thus, the two distinctly different sets of plant damage symptoms (i.e., burning versus stippling) are associated with different combinations of tactics within cell rupture feeding, applied in different plant tissues. Lacerate-and-flush and

lance-and-ingest are performed to varying degrees by both stipplers and burners. However, stipplers perform sawing laceration during long duration, usually interveinal probes into mesophyll. Burners perform pulsing laceration during short duration, primarily vascular probes.

Within the typhlocybines, most species are stipplers, including most members of Empoascini (Table 1). Therefore, sawing laceration is probably the ancestral typhlocybine behavior. We hypothesize that an empoascine ancestor evolved pulsing laceration and then subsequent speciation led to a radiation of a number of empoascine burner species. In other words, burning may be monophyletic in one branch of Empoascini. This idea is supported by the finding that the burner species can partially perform sawing laceration (the main stippling behavior) if needed to survive, but the stippler species apparently cannot perform pulsing laceration (the main burning behavior).

In contrast, burning seems to have evolved independently among other, nonempoascine typhlocybines, and even nontyphlocybine auchenorrhynchans. The most drastic case is seen in the delphacid planthoppers *N. lugens* and *S. furcifera*. At present, there is no evidence of cell rupture feeding in any planthopper, which suggests that the mechanism of hopperburn initiation by these species is more related to salivary physiology than to feeding behavior.

Finally, the stylet penetration tactics of *Empoasca* spp. demonstrate that auchenorrhynchans can exhibit considerable plasticity in their repertoire of feeding behaviors and that the differences between the two hemipteran feeding strategies may not be as clear-cut as was once believed. The frequently observed R waveform may prove to represent flushing by sheath-feeding auchenorrhynchans. Perhaps such mesophyll ingestion via flushing through a salivary sheath is an intermediate behavior between sheath feeding and cell rupture feeding, similar to lance-and-ingest. The importance of such behavioral flexibility is most apparent when we consider the roles of stylet penetration tactics in hopperburn initiation by empoascines.

Tactics are Performed in Different Proportions on Different Host Plants

One of the most unique findings from the work of Backus and colleagues on *Empoasca* feeding is that the stylet penetration tactics are usually performed by the same insect species in varying proportions on different host plants. Thus, the burner *Empoasca* spp. apparently can assess the phagostimulants of a potential host plant, and then choose within their behavioral repertoire of penetration tactics to best exploit that particular plant. This is yet another level of behavioral plasticity never documented before for hemipterans, which more often are thought to have highly stereotypical feeding behavior (77).

Tactic switching by *Empoasca* spp. is especially striking among host plant species. For example, on alfalfa, susceptible cv. 'Ranger,' adult females of both *E. fabae* (8) and *E. kraemeri* (E.A. Backus, unpublished data) prefer to feed on the stems. Kabrick & Backus (38) found that adult female *E. fabae* on alfalfa spend

more than 80% of their total probing duration in continuous bouts of pulsing laceration, about 15% in lance-and-ingest (called I_c therein), and less than 5% in lacerate-and-flush (called I_b or single-cell puncturing therein). In contrast, when both species feed on a susceptible genotype of common bean (cv. Porrillo Sintetico), they prefer leaves, feeding about half the time on veins and the other half interveinally. About 43% of total probing duration is spent in pulsing laceration, 25% in lance-and-ingest, and 32% in lacerate-and-flush (12, 65).

Although less striking, this switching of tactics also occurs among genotypes of the same host plant, as demonstrated by a study of *E. kraemeri* on five genotypes of common bean from CIAT (Centro Internationale Agricultura Tropical, Cali, Colombia) (65, 67). Hypersusceptible (BAT 41) and susceptible (Porrillo Sintetico) genotypes were compared with moderately tolerant (EMP 84) and highly tolerant (EMP 385 and EMP 392) genotypes. While pulsing laceration was always performed on all genotypes, its proportion of total probing time decreased from 44% on the hypersusceptible genotype to 32%–34% on the highly tolerant genotypes. Proportions of lacerate-and-flush (called single-cell puncturing in Reference 12 and cell rupturing in Reference 67) were significantly increased on the tolerant cultivars, especially EMP 385 and EMP 392 (to 42%–43% of probing), compared with both the susceptible genotypes (26%–28%). Lance-and-ingest (67; called I_c in Reference 12) also varied significantly, from 12% to 24% of probing.

SALIVARY PHYSIOLOGY ASSOCIATED WITH HOPPERBURN

Types and Composition of Hemipteran Saliva in General

Salivary composition of many hemipteran species has been broadly studied and is reviewed elsewhere (57, 59). Therefore, we only summarize it herein. Two broad categories of saliva are presently thought to be made by all hemipterans. The first is watery saliva, which is composed of digestive, hydrolyzing, and cell wall–degrading enzymes. Composition is highly variable among species (57) but can include carbohydrases such as amylase (14, 31, 32), pectinases (33, 48) and cellulase (13), lipase and protease (15), hydrolases (50, 51), and alkaline phosphatase (25). Protease is commonly found in salivary glands of many phytophagous piercing-sucking insects, but especially in those using the cell rupture feeding strategy (34). In addition to these hydrolytic enzymes, watery saliva usually also contains oxidative enzymes (23, 73) such as catechol oxidase (51, 57, 58), polyphenol oxidase (71), and peroxidase (51). Watery saliva disperses readily from the site of injection and is too diffuse to be stained with conventional histological stains.

The second type of saliva is sheath saliva, which is composed of lipoproteins, phospholipids, and conjugated carbohydrates. It is rapidly solidified by oxidizing enzymes following ejection from the stylets, forming a salivary sheath that surrounds the stylets as they penetrate the plant (57, 70). The density of its lipoprotein structure readily allows conventional histological staining.

In most hemipterans, both types of saliva can be discharged during stylet penetration. However, the relative amounts of each type secreted, and their composition, vary both by species and by feeding strategies used. It is thought that all hemipterans secrete watery saliva with every probe. In addition, sheath feeders also secrete sheath saliva during every probe [although such reputed fidelity has been tested convincingly in only a few species (38)]. The sheaths of sheath feeders (i.e., true sheaths) are quite solid. Though their durability in plants has seldom been tested, Bennett (9) found that sheaths of beet leafhopper, *Circulifer tenellus* (Baker), remained intact in the plant up to 34 days, and those of glassy-winged sharpshooter, *Homalodisca coagulata* (Say), lasted at least 80 days (E.A. Backus & J. Habibi, unpublished data). In contrast, cell rupture feeders secrete little sheath saliva, usually in disconnected, globular deposits (38, 63). Also, their sheath saliva appears semiliquid and gelatinous; its durability in the plant is much lower than that of true sheath feeders (Figure 3). For example, in a time course study of *E. fabae* feeding on alfalfa by Ecale & Backus (19), deposits of stainable saliva, frequently seen at one-half day after a pulse of feeding, gradually diminished in size

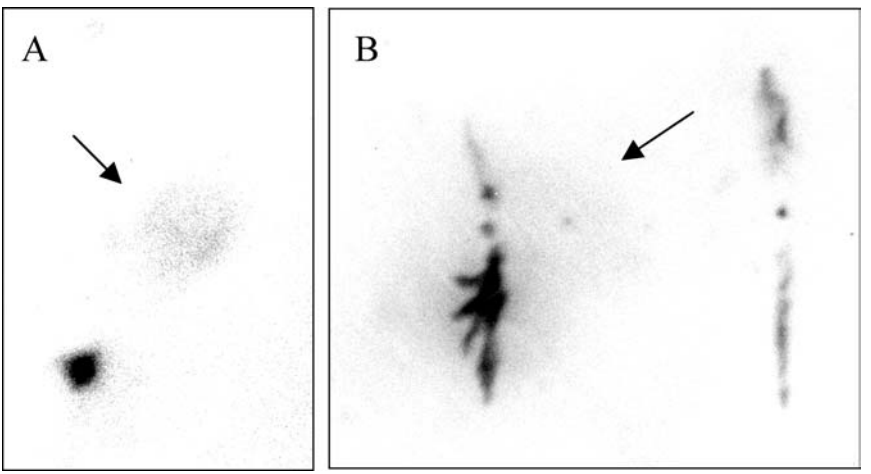

Figure 3 *E. fabae* pseudosheaths and deposits of watery saliva in viscous artificial diet (4% agarose, 5% sucrose) (29). Image is not blurry, rather the pseudosheaths themselves are indistinct and tenuous. (*A*) Globular deposit of pseudosheath material (darkly stained by Bradford reagent) at the end of a channel cut in the artificial diet fed upon by a male adult. Note faint billow of watery saliva (*arrow*). (*B*) Two fan-shaped, tenuous pseudosheaths by nymphal leafhoppers. Visual aspect is straight-on so that fan branches appear to come out of the page toward the reader. Note faint halo of watery saliva around the left fan (*arrow*). Pseudosheaths often were found in each channel of a fan in artificial diet, although rarely so in plants. This suggests they were either secreted more often in artificial diet or preserved from degradation by plant enzymes when left in diet. Data from Reference 17.

and number until they were no longer visible by day four. This pattern suggests that deposits were eventually dissolved by enzymatic processes in the plant. Also pseudosheaths (larger deposits of gelatinous sheath saliva) often appear to be intercellular (38, 69), an unlikely position for auchenorrhynchan sheaths because these insects probe intracellularly. This position may be caused by expansion of vascular cambial cells after saliva deposition, which pushes the gelatinous saliva into newly created intercellular spaces.

The Role of Salivary Composition in Stippling Versus Burning

Almost nothing is known about functional categories of salivary proteins of typhlocybines and/or hopperburners. In an early study of *E. fabae* saliva (10), invertase, amylase, and protease were colorimetrically detected in homogenized salivary glands, although not in artificial diet solutions fed upon for 24 to 96 h by *E. fabae* nymphs. Modern electrophoretic techniques were used more recently to show that denatured protein profiles of secreted (probably mostly watery) saliva from both *E. fabae* and *E. abrupta* were diverse and complex. Two findings are relevant to hopperburn initiation. First, protein profiles were different for the two species, even when insects consumed the same host plant species (28). Stippling versus burning symptoms thus may be due not only to feeding behavior differences, but also to differing salivary composition of stipplers and burners. Therefore, some of the earliest theories that hopperburn is initiated by toxic saliva may be partially supported (35).

Second, salivary protein profiles of both *E. fabae* and *E. abrupta* were affected by the recent dietary history of the individual insect. For example, four extra protein bands were seen when *E. fabae* fed on broad bean compared with simple artificial diet; even greater differences were seen on common bean (28). It is unknown whether this result is due to leftover protein from prior meals being recycled into the saliva; however, Miles (59) speculates this would be more likely for amino acids than proteins. Alternatively, induction of new, unique protein synthesis could be occurring perhaps to counter inducible plant-defensive compounds. In support of this idea, the salivary protein profile of *Lygus hesperus* Knight, another phytophagous cell rupture feeder, also varied by dietary history, whereas that of an entomophagous cell rupture feeder, *Podisus maculiventris* (Say), did not. This variation in salivary protein profile based on dietary history provocatively suggests that saliva of varying toxicity could be secreted by the same insect on different host plants, further exacerbating the differences in symptoms between stipplers and burners, or for burners among plant species.

The Role of Wounding Versus Saliva in Hopperburn Initiation

Two primary insect stimuli are likely to occur to the plant during pulsing laceration, i.e., mechanical wounding and copious (unstainable) watery salivation (Figure 2) into the wounded but still living cells. Ecale & Backus (19) separately

examined the effects of each of these stimuli from *E. fabae* feeding in alfalfa (mostly pulsing laceration), and then recombined them to determine which would initiate the localized cellular abnormalities in the vascular tissues associated with hopperburn. For the salivary stimulus alone, fresh salivary glands were implanted under minimally damaged epidermis of alfalfa stems. For the mechanical stimulus alone, ground, sharpened minuten pins were punctured into alfalfa stems to the same depths as stylets during probing. To recombine the stimuli, punctures were made through freshly dissected salivary glands. Salivary gland implantation did not trigger abnormalities, which suggests that saliva could not diffuse inward to the vascular bundles. Mechanical punctures alone triggered some abnormalities, consistent with normal plant wound responses (39, 46), that resembled those of mild, early-stage hopperburn (20, 38). In contrast, when the same number of punctures was made through salivary glands, extreme cellular abnormalities resulted.

It was concluded that a combination of mechanical and salivary stimuli is necessary to trigger the hopperburn cascade in alfalfa, and it was proposed that hopperburn is a saliva-enhanced wound response (20). Thus, the mechanical stimulus of the lacerating stylets is the initial trigger of wound responses that begin in a normal, organized fashion. But factors within the saliva probably cause derailment of the normal wound response.

The preponderance of evidence to date supports that salivary effects are localized to the tissue immediately surrounding the probing site. Cellular hypertrophy (whose appearance and effects are described further below) can be caused by either watery saliva or large deposits of pseudosheath saliva from lance-and-ingest (62). Hypothetically, lance-and-ingest might cause small amounts of watery saliva to be translocated through a lanced sieve element to distant sites away from the vicinity of probing. However, results from calculations of the Stylet Penetration Index (SPI) (67) suggest that effects from such putatively translocated saliva are probably only a minor contribution to yield reductions. Thus, the old theory that *Empoasca* saliva initiates effects at long distances from the site of probing (35) is not presently supported.

In contrast, in the case of hopperburning delphacids, saliva alone is implicated in hopperburn initiation in the absence of clearly lacerating feeding behavior. Direct damage localized to the site of feeding has been documented and well studied for many years in certain aphid species such as greenbug, *Schizaphis graminum* (Rondani). Evidence supports that polygalacturonase and other cell wall–degrading enzymes in aphid saliva trigger responses that lead to localized chlorosis (13, 49). That hopperburn symptoms from planthopper feeding appear more systemic and less local could imply entry of saliva into the vascular system and its rapid transport away from the site of feeding, as has been shown for the direct-damaging spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (51). This is plausible for auchenorrhynchans, since a vascular salivation waveform, called waveform no. 4, has been found in a study of *C. mbila* (45).

SUMMARY OF PLANT RESPONSES ASSOCIATED WITH HOPPERBURN

Plant Responses Due to Pulsing Laceration

The evidence supports that, to a large extent, pulsing laceration causes the most drastic, generalized hopperburn symptoms of stunting and chlorosis that occur on all host plants. All burners studied to date perform pulsing laceration on all host plants to some degree. Pulsing laceration is almost the only behavior performed in some burner–host plant systems, such as adult *E. fabae* on alfalfa. Likewise, hopperburn symptoms on alfalfa are straightforwardly limited to chlorosis and stunting.

Several studies have been published on the plant responses triggered by pulsing laceration of alfalfa stems by *E. fabae* (18–21) and of common bean veins by *E. kraemeri* (66). These results will be more extensively described in the companion paper in preparation. In summary, Ecale & Backus (18) developed a means of standardizing and concentrating the amount of probing to a small area of alfalfa stem. They then performed a time course study of plant damage initiated by standardized probing (19, 21). A few minutes of primarily pulsing laceration initiates a cascade of plant physiological and anatomical changes requiring a full eight days to complete.

The cascade started as a normal wound response in which damaged phloem cells were crushed and pushed aside by orderly, columnar cell division of adjacent cambial cells. By day two, the phloem had become completely blocked, which explains in part the finding of reduced translocation by Nielson et al. (61). In addition, the normal wound response had become derailed and cells had arisen in atypical planes of division. Also, immature xylem cells had collapsed and no new cells matured. At day four, phloem and xylem cells had proliferated greatly and in irregular shapes, similar to callus tissue. This proliferative growth was so severe that it resembled the nutritive (or reaction) tissues of plant galls. In the absence of sheath saliva, one could probably guess that laceration had occurred by observing such cellular abnormalities. By day eight, phloem cells looked reorganized, but in fact they were mostly nonfunctional. Instead, wound phloem bypasses had restored translocation around the permanently nonfunctional area (21). Xylem cells had been significantly reduced in both size and number, causing a 48% reduction in xylem area (19). Later work showed that at day two pulsing laceration causes cellular abnormalities in bean veins similar to those in alfalfa stems (66).

Overall, the negative impacts on phloem and xylem from the saliva-enhanced wound response triggered by pulsing laceration have far-reaching, systemic effects on the plant that require time and energy to heal, if they heal at all. The net effects from pulsing laceration of a vascular bundle are (*a*) permanent reduction in water translocation through the xylem, and (*b*) a temporary blockage of photoassimilate translocation through phloem sieve elements, which is eventually healed through an energy- and time-consuming process. During that process, however, this region

undoubtedly becomes an energy sink, rather than a region through which photoassimilates are transported from source leaves to sink regions. As will be described more fully in the companion paper, these vascular effects on xylem and phloem cause shutdown of photosynthesis, via a mechanism that is poorly understood but under active study. Stomatal resistance may play a key role, but some feedback inhibition of photosystems by accumulation of photosynthates may occur as well (61). These effects probably cause both stunting and chlorosis.

Plant Responses Due to Lacerate-and-Flush and Lance-and-Ingest

Lacerate-and-flush and lance-and-ingest are performed to varying degrees on different hosts. On common bean, both *E. fabae* and *E. kraemeri* spend at least half their probing time in lacerate-and-flush or lance-and-ingest. Common bean also exhibits the less severe symptoms of leaf curling and necrosis, in addition to stunting and chlorosis, which suggests that leaf curling and necrosis are caused, at least in part, by the latter two tactics.

Hopperburn initiation is more complex in common bean than in alfalfa, probably involving the combined effects of all three tactics. Lacerate-and-flush leads to physical removal of cell contents, causing cell shrinkage and therefore leaf wrinkling. Differential cell rupturing on one side of the leaf, frequently observed (62), probably contributes to leaf curling. Both effects cause reduction of net photosynthetic capabilities of mesophyll cells, exacerbating stunting and yield reductions. Therefore, lacerate-and-flush is probably moderately damaging.

Lance-and-ingest can stimulate expansion of the cell layer proximate to the stylet insertion point, probably in reaction to pseudosheath saliva (62). Combined with differential cell shrinkage by lacerate-and-flush, this expansion probably contributes to the bean- and potato-specific symptom of leaf curling. In turn, curling reduces the production of photoassimilates and might also introduce small quantities of watery saliva into the phloem translocation stream (see Salivary Physiology Associated with Hopperburn, above).

IMPLICATIONS FOR HOST PLANT RESISTANCE

Could knowledge of *Empoasca* feeding behaviors performed on a host plant partly explain and predict the degree of hopperburn damage that would occur? If so, then EPG monitoring could be used to segregate resistant and susceptible host plants. Plant responses, either damaging or healing/compensatory, could then explain the remaining portion of hopperburn damage not accounted for by the initiating behavior. To answer this question, EPG monitoring was performed at CIAT with *E. kraemeri* on five susceptible or tolerant genotypes of common bean, and accompanied by a standard field trial of the same genotypes under natural infestation pressures (65–67). Data on bean yield and yield components were taken for the field study, and waveform data from the EPG monitoring were measured and

extensively analyzed for any possible correlations (67). SPI was devised and compared with a field yield-based resistance index (RI) developed by researchers at CIAT (42, 43). Results are extensively discussed in Reference 67.

In brief, we categorized every probe recorded as belonging to one of *E. kraemeri*'s three stylet penetration tactics and then calculated numbers and durations of probes per insect of each tactic. These six variables were subjected to a principal components analysis, and three new factors were created that accounted for 99.93% of overall variance (67). The variables that constituted each factor closely matched the variables of the three tactics, and so were designated as scores for the performance of each tactic on a genotype. A linear equation relating the tactic scores to one another was then derived, on the basis of the degree of damage caused by each penetration tactic (67). The equation generated a single value, the SPI, for each genotype.

For three of the five studied genotypes (BAT 41, EMP 385, and EMP 392), the SPI and RI values were virtually identical (Figure 4). But even for the dissimilar genotypes (Porrillo Sintetico and EMP 84), the relative difference between index values explained more about their resistance traits than had been understood before. These two were considered borderline resistant genotypes by CIAT researchers. EPG results suggest that Porrillo Sintetico stimulates damaging feeding behavior, but preserves yield (67) by producing less injurious (or more healing,

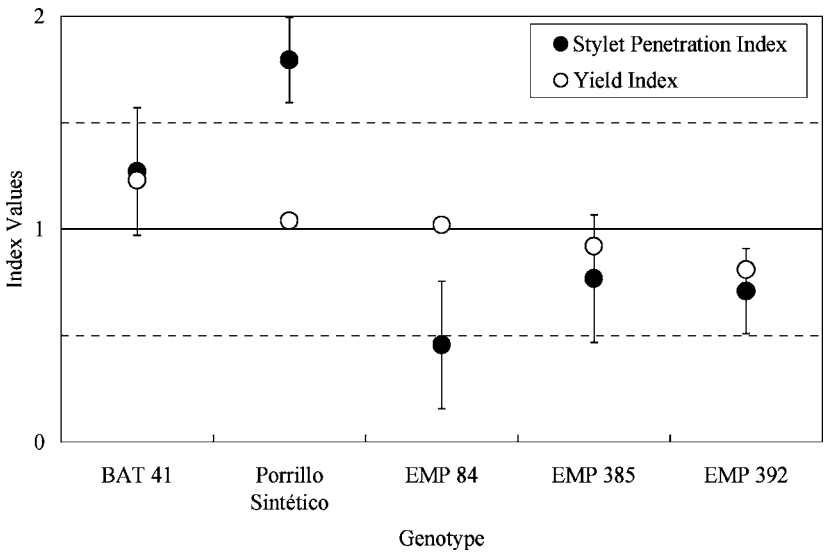

Figure 4 Comparison of values for the SPI and RI of *E. kraemeri* for five genotypes of *P. vulgaris*. For the SPI, each dot represents an average value (±SEM, $N = 30$). From Serrano et al. (67). Reprinted with permission from the *Journal of Economic Entomology*, Entomological Society of America.

even compensatory) plant responses (66). In contrast, EMP 84 stimulates a switch to less damaging feeding, but is probably highly sensitive to even that low level of laceration and responds negatively to it. The two sets of effects balance out to yield two borderline RI values, but by entirely different mechanisms (67). This SPI formula has also been applied to *E. fabae* feeding on alfalfa and potato and successfully predicted the degree to which hopperburn initiation is worsened by drought or mineral stress (3; E.A. Backus, unpublished data).

Finally, a recent preliminary observation deserves mention. In the published formula for the SPI, equal weight is given to each of the tactic scores, even though each tactic is not performed equally. However, if one multiplies each tactic score by the percentage proportion of total feeding represented by that tactic, the SPI values for all five genotypes become virtually identical to the values for the RI. These new values essentially duplicate the field results (67). A similar modification was used for an RI, on the basis of the SPI, that duplicated results from field cultivar tests with laboratory bioassay results (68). Thus, if these preliminary results prove true, the original SPI of Serrano et al. (67) overweighted the nonlaceration behaviors, making the index supersensitive to decreases in hopperburn-initiating pulsing laceration. Such overweighting would be useful to identify plants with unusual traits for breeding. Alternatively, one could duplicate field results more quickly and cost-effectively (67) using a modified, weighted SPI.

Therefore, waveform categorization by stylet penetration tactic allowed, for the first time in any EPG monitoring research, all probing data to be distilled to a single number for each host plant for unambiguous comparisons among genotypes. The SPI can be used in concert with or in lieu of traditional germplasm screening methods (67). In either case, the SPI demonstrates the applied value of understanding the fundamental feeding biology of hopperburning species, in both explaining and predicting hopperburn initiation.

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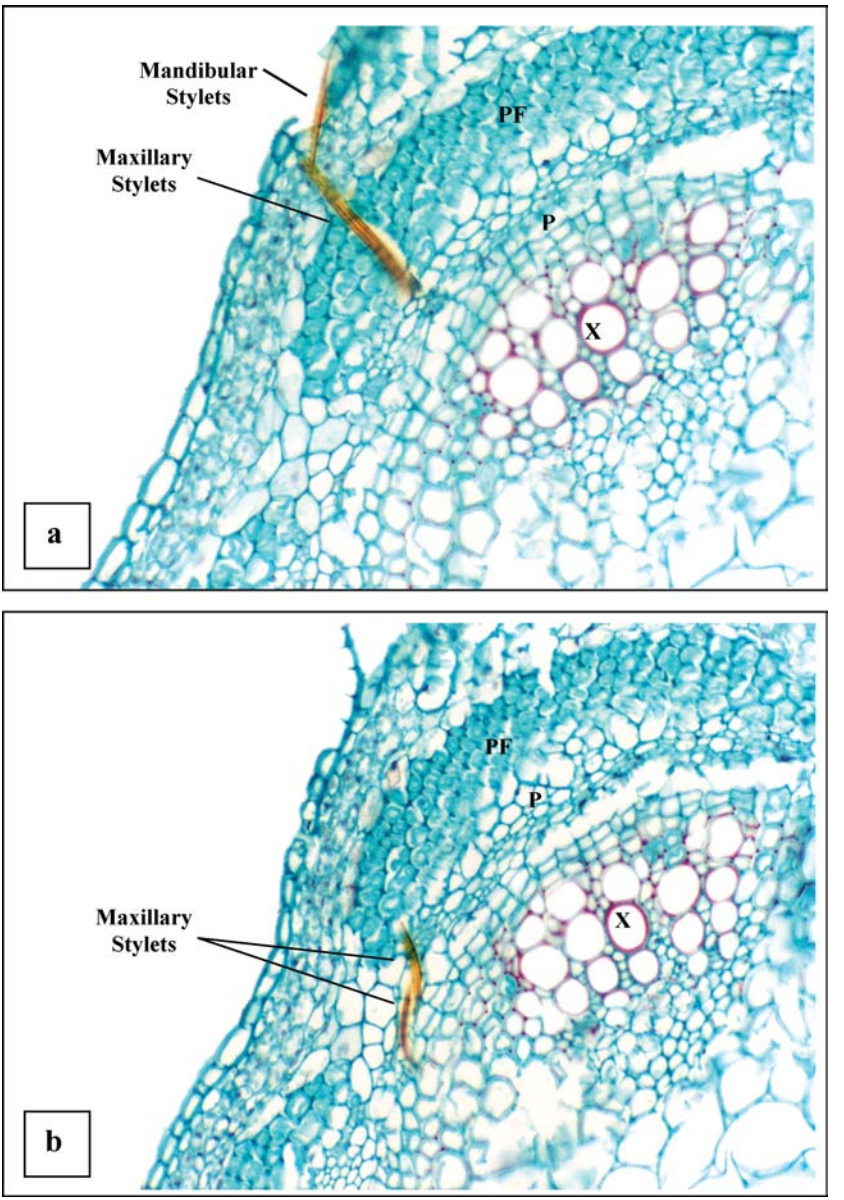

Figure 2 Two adjoining sections through the stylets of *E. fabae* during I_a on alfalfa. (*a*) The middle portion of the maxillary stylets bundle is present. The mandibular stylet has been dislodged from its normally shallow position. (*b*) The tips of the maxillary stylets, seen splayed apart from one another, terminating in the phloem and interfascicular parenchyma. P, phloem tissue; PF, phloem fiber cells; X, xylem tracheary elements. Magnification 10×. From X. Ni, unpublished data.

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